

CLAIMS

1. An isolated nucleic acid encoding a functional MC4R, or the complement thereof, said nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of SEQ ID NO:1;

(b) a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of SEQ ID NO:1;

(c) a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of the feline MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1762**; and

(d) a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of the feline MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1762**, with the proviso that said functional MC4R is not human, porcine, murine, rat or chicken.

2. An isolated nucleic acid encoding a functional MC4R, or the complement thereof, said nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence which hybridizes under conditions of high stringency to the coding region of SEQ ID NO:1;

(b) a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of SEQ ID NO:1;

(c) a nucleotide sequence which hybridizes under conditions of high stringency to the coding region of the feline MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1762**; and

(d) a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of the feline MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

3. An isolated nucleic acid comprising a nucleotide sequence that:

(a) encodes a polypeptide according to SEQ ID NO:3; or

(b) encodes a polypeptide encoded by the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

4. The isolated nucleic acid of Claim 3, wherein said nucleic acid has a nucleotide sequence according to SEQ ID NO:1 or the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

5. An isolated nucleic acid comprising a nucleotide sequence having more than 87.4% identity to SEQ ID NO:1 or the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

6. An isolated nucleic acid comprising a nucleotide sequence encoding a polypeptide having more than 98.2% identity to SEQ ID NO:3 or the polypeptide encoded by the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

7. An isolated nucleic acid comprising a nucleotide sequence encoding an ECD of a feline MC4R corresponding to amino acids 1-46, 96-123, 186-190, or 268-279 of SEQ ID NO:3 or of the polypeptide encoded by the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

8. An isolated nucleic acid comprising a nucleotide sequence encoding a CD of a feline MC4R corresponding to amino acids 70-76, 146-165, 212-244, or 302-333 of SEQ ID NO:3 or of the polypeptide encoded by the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**.

9. An isolated nucleic acid encoding a functional MC4R, or the complement thereof, said nucleic acid comprising a nucleic acid selected from the group consisting of:

(a) a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of SEQ ID NO:2;

(b) a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of SEQ ID NO:2;

(c) a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of the canine MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1761**; and

(d) a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of the canine MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1761**,

with the proviso that said functional MC4R is not human, porcine, murine, rat or chicken.

10. An isolated nucleic acid encoding a functional MC4R, or the complement thereof, said nucleic acid comprising a nucleic selected from the group consisting of:

(a) a nucleotide sequence which hybridizes under conditions of high stringency to the coding region of SEQ ID NO:2;

5 (b) a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of SEQ ID NO:2;

(c) a nucleotide sequence which hybridizes under conditions of high stringency to the coding region of the canine MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1761**; and

10 (d) a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of the canine MC4R as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

11. An isolated nucleic acid comprising a nucleotide sequence that:

(a) encodes a polypeptide according to SEQ ID NO:4; or

15 (b) encodes a polypeptide encoded by the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

12. The isolated nucleic acid of Claim 11, wherein said nucleic acid has a nucleotide sequence according to SEQ ID NO:2 or the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

20 13. An isolated nucleic acid comprising a nucleotide sequence having more than 81.3% identity to SEQ ID NO:2 or the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

14. An isolated nucleic acid comprising a nucleotide sequence encoding a polypeptide having more than 98.1% identity to SEQ ID NO:4 or to the polypeptide encoded by the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

25 15. An isolated nucleic acid comprising a nucleotide sequence encoding an ECD of a canine MC4R corresponding to amino acids 1-46, 98-124, 187-191, or 268-279 of SEQ ID NO:4 or of the polypeptide encoded by the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

30 16. An isolated nucleic acid comprising a nucleotide sequence encoding a CD of a canine MC4R corresponding to amino acids 69-77, 147-163, 216-244, or 302-333 of SEQ ID NO:4 or of the polypeptide encoded by the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.

35

17. A nucleotide vector comprising the nucleic acid of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.
18. An expression vector comprising the nucleic acid of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 in operative association with a nucleotide regulatory element that controls expression of the polypeptide encoded by said nucleotide sequence.
19. A genetically engineered host cell comprising the nucleic acid of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.
20. A genetically engineered host cell comprising the nucleic acid of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 wherein said nucleic acid is in operative association with a nucleotide regulatory element that controls expression of said nucleotide sequence in the host cell.
21. A substantially pure polypeptide encoded by the nucleic acid of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.
22. A substantially pure polypeptide comprising the amino acid sequence of :
- (a) SEQ ID NO:3;
- (b) SEQ ID NO:4;
- (c) the feline MC4R clone as deposited with the ATCC and having the ATCC Accession NO. **PTA-1762**;
- (d) the canine MC4R clone as deposited with the ATCC and having the ATCC Accession NO. **PTA-1761**;
- (e) an ECD of a feline MC4R corresponding to amino acids 1-46, 96-123, 186-190, or 268-279 of SEQ ID NO:3 or of the polypeptide encoded by the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**;
- (f) an ECD of a canine MC4R corresponding to amino acids 1-46, 98-124, 187-191, or 268-279 of SEQ ID NO:4 or of the polypeptide encoded by the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**;
- (g) a CD of a feline MC4R corresponding to amino acids 70-76, 146-165, 212-244, or 302-333 of SEQ ID NO:3 or of the polypeptide encoded by the feline MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1762**; or
- (h) a CD of a canine MC4R corresponding to amino acids 69-77, 147-163, 216-244, or 302-333 of SEQ ID NO:4 or of the polypeptide encoded by the canine MC4R clone as deposited with the ATCC and having ATCC Accession No. **PTA-1761**.
23. An antibody that immunospecifically binds the polypeptide of Claim 21.
24. A method for producing a recombinant polypeptide, comprising:

(a) culturing a host cell transformed with the expression vector of Claim 18 and which expresses the recombinant polypeptide; and

(b) recovering the recombinant polypeptide from the cell culture.

- ✓ 25. A composition comprising the polypeptide of Claim 21 and a carrier.
- 5 ✓ 26. A method for detecting a polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 in a sample, comprising:

(a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex; and

(b) detecting the complex,
10 so that if a complex is detected, a polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 is detected.

✓ 27. A method for detecting a polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 in a sample, comprising:

(a) contacting the sample under stringent hybridization conditions with
15 nucleic acid primers that anneal to a polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 under such conditions; and

b) amplifying the annealed polynucleotides,
so that if a polynucleotide is amplified, the polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 is detected.

20 28. The method of Claim 27, wherein the polynucleotide is an RNA molecule that encodes a functional MC4R, and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.

29. A method for identifying a compound that binds to the polypeptide of Claim 21, comprising:

25 (a) contacting a compound with the polypeptide of Claim 21 for a time sufficient to form a polypeptide/compound complex; and

b) detecting the complex,
so that if a polypeptide/compound complex is detected, a compound that binds to a polypeptide of Claim 19 is identified.

30 30. A method for identifying a compound that binds to the polypeptide of Claim 21, comprising:

(a) contacting a compound with a polypeptide of Claim 21, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in said cell; and

- b) detecting the complex by detecting reporter gene sequence expression,

so that if a polypeptide/compound complex is detected, a compound that binds to a polypeptide of Claim 21 is identified.

- 5 ✓ 31. A method of modulating activity of the polypeptide of Claim 21, comprising contacting a cell that expresses the polypeptide with a compound that modulates activity of the polypeptide for a time sufficient to modulate said activity.

32. A method for screening and identifying antagonists of MC4R, comprising:

- 10 (a) contacting a cell line that expresses the polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 with a test compound in the presence of an MC4R agonist; and

(b) determining whether the test compound inhibits the binding and cellular effects of the MC4R agonist on the cell line,

15 in which antagonists are identified as those compounds that inhibit both the binding and cellular effects of the MC4R agonist on the cell line.

33. A method for screening and identifying agonists of MC4R, comprising:

(a) contacting a cell line that expresses the polynucleotide of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 with a test compound in the presence and in the absence of an MC4R agonist;

20 (b) determining whether, in the presence of the MC4R agonist, the test compound inhibits the binding of the MC4R agonist to the cell line; and

(c) determining whether, in the absence of the MC4R agonist, the test compound mimics the cellular effects of the MC4R agonist on the cell line,

25 in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of the MC4R agonist on the cell line.

34. The method of Claim 33 in which the cell line is a genetically engineered cell line.

35. A method for screening and identifying antagonists of MC4R comprising:

30 (a) contacting the polypeptide of Claim 21 with a random peptide library such that the polypeptide will recognize and bind to one or more peptide species within the library;

(b) isolating the polypeptide/peptide combination;

(c) determining the sequence of the peptide isolated in step (b); and

35 (d) determining whether the test compound inhibits the binding and cellular effects of an MC4R agonist,

in which antagonists are identified as those peptides that inhibit both the binding and cellular effects of the MC4R agonist.

36. A method for screening and identifying agonists of MC4R comprising:

- 5 (a) contacting the polypeptide of Claim 21 with a random peptide library such that the polypeptide will recognize and bind to one or more peptide species within the library;
- (b) isolating a polypeptide/peptide combination;
- (c) determining the sequence of the peptide isolated in step (b); and
- (d) determining whether, in the absence of a MC4R agonist, the peptide
- 10 mimics the cellular effects of the MC4R agonist,

in which agonists are identified as those peptides that inhibit the binding of the MC4R agonist to a MC4R polypeptide but mimic the cellular effects of the MC4R agonist.

37. A method of modulating activity of the polypeptide of Claim 21, comprising

15 contacting the polypeptide with a compound that modulates activity of the polypeptide for a time sufficient to modulate said activity.

38. A method of modulating the endogenous enzymatic activity of MC4R in an animal comprising administering to the animal an amount of an MC4R ligand effective to modulate said endogenous enzymatic activity.

20 39. The method of Claim 38, wherein the animal is a cow, a pig, a goat, a sheep, a horse, a dog, or a cat.

40. The method of Claim 38 in which the ligand to said MC4R receptor is an MC4R agonist.

25 41. The method of Claim 38 in which the ligand to said MC4R receptor is an MC4R antagonist.

42. The antagonist of Claim 41 that is a monoclonal antibody that immunospecifically binds to an epitope of said MC4R.

43. The method of Claim 38 in which the enzymatic activity of said MC4R is increased.

30 44. The method of Claim 38 in which the enzymatic activity of said MC4R is decreased.

45. A transgenic animal in which the nucleic acid of Claim 1 or 9 is an expressed transgene contained in the genome of the animal.

35 46. A transgenic animal in which expression of genomic sequences encoding the polypeptide of Claim 21 is prevented or repressed.

47. A method for modulating the appetite and/or metabolic rate of an animal comprising administering to the animal an effective amount of an MC4R ligand.

48. The method of Claim 47, wherein the animal has an appetite-related or metabolic disorder.

5 49. The method of Claim 48 wherein the disorder causes, is caused by, or is characterized by a reduction in appetite, feeding behavior or body weight, or an increase in metabolic rate, and the ligand is an MC4R antagonist.

50. The method of Claim 49 wherein the disorder causes, is caused by, or is characterized by an increase in appetite, feeding behavior, or body weight, or a decrease in metabolic rate, and the ligand is an MC4R agonist.

51. The method of Claim 49 wherein the animal is a *post partum* sow or dairy cow.

52. The method of Claim 48 wherein the animal is a companion animal.

53. The method of Claim 48 wherein the animal is a livestock animal.

15 54. The method of Claim 48 wherein the animal is a poultry animal.

55. The method of Claim 49 wherein the animal suffers from shipping or crowding stress.

56. The method of Claim 49 wherein the animal is lactating.

57. The method of Claim 47 wherein the animal is gravid.

20 58. The method of Claim 49 wherein the disorder is cachexia, anorexia or weaning-induced inappetance and growth lag.

59. The method of Claim 49 wherein the disorder is a metabolic disorder.

60. The method of Claim 59 wherein the metabolic disorder is diabetes.

61. The method of Claim 49 wherein the disorder is a disease.

25 62. The method of Claim 61 wherein the disease is cancer, renal failure, cardiac disease, endotoxemia, fever, hepatic lipidosis, infection or inflammation.

63. The method of Claim 50 wherein the animal is obese.

64. The method of Claim 47 wherein the MC4R ligand is part of a pharmaceutical composition.

30 65. A method of increasing the reproductive performance of an animal comprising administering to the animal an effective amount of an MC4R ligand.

66. A method of increasing the growth performance of an animal comprising administering to the animal an effective amount of an MC4R ligand.

35 67. The method of Claim 47 wherein the ligand is part of a pharmaceutical composition.

- | Variable | Mean | Standard Deviation | Minimum | Maximum |
|--------------------------------|-------|--------------------|---------|---------|
| Age | 34.5 | 10.2 | 21 | 55 |
| Gender | 0.5 | 0.5 | 0 | 1 |
| Marital Status | 0.6 | 0.5 | 0 | 1 |
| Education | 12.5 | 1.5 | 9 | 16 |
| Income | 15000 | 5000 | 5000 | 30000 |
| Health | 0.8 | 0.2 | 0 | 1 |
| Smoking | 0.3 | 0.5 | 0 | 1 |
| Drinking | 0.2 | 0.4 | 0 | 1 |
| Exercise | 0.4 | 0.5 | 0 | 1 |
| Stress | 0.6 | 0.5 | 0 | 1 |
| Sleep | 0.7 | 0.3 | 0 | 1 |
| Appetite | 0.8 | 0.2 | 0 | 1 |
| Mood | 0.9 | 0.1 | 0 | 1 |
| Energy | 0.7 | 0.3 | 0 | 1 |
| Concentration | 0.8 | 0.2 | 0 | 1 |
| Memory | 0.9 | 0.1 | 0 | 1 |
| Emotion | 0.7 | 0.3 | 0 | 1 |
| Behavior | 0.8 | 0.2 | 0 | 1 |
| Thought | 0.9 | 0.1 | 0 | 1 |
| Feeling | 0.8 | 0.2 | 0 | 1 |
| Perception | 0.9 | 0.1 | 0 | 1 |
| Attention | 0.8 | 0.2 | 0 | 1 |
| Intuition | 0.7 | 0.3 | 0 | 1 |
| Imagination | 0.6 | 0.4 | 0 | 1 |
| Reasoning | 0.9 | 0.1 | 0 | 1 |
| Logic | 0.8 | 0.2 | 0 | 1 |
| Analysis | 0.7 | 0.3 | 0 | 1 |
| Synthesis | 0.6 | 0.4 | 0 | 1 |
| Evaluation | 0.9 | 0.1 | 0 | 1 |
| Problem Solving | 0.8 | 0.2 | 0 | 1 |
| Decision Making | 0.7 | 0.3 | 0 | 1 |
| Communication | 0.9 | 0.1 | 0 | 1 |
| Interpersonal Skills | 0.8 | 0.2 | 0 | 1 |
| Teamwork | 0.7 | 0.3 | 0 | 1 |
| Leadership | 0.6 | 0.4 | 0 | 1 |
| Management | 0.9 | 0.1 | 0 | 1 |
| Organization | 0.8 | 0.2 | 0 | 1 |
| Planning | 0.7 | 0.3 | 0 | 1 |
| Execution | 0.9 | 0.1 | 0 | 1 |
| Monitoring | 0.8 | 0.2 | 0 | 1 |
| Evaluation | 0.7 | 0.3 | 0 | 1 |
| Feedback | 0.9 | 0.1 | 0 | 1 |
| Improvement | 0.8 | 0.2 | 0 | 1 |
| Innovation | 0.7 | 0.3 | 0 | 1 |
| Creativity | 0.6 | 0.4 | 0 | 1 |
| Adaptability | 0.9 | 0.1 | 0 | 1 |
| Resilience | 0.8 | 0.2 | 0 | 1 |
| Stress Management | 0.7 | 0.3 | 0 | 1 |
| Emotional Regulation | 0.9 | 0.1 | 0 | 1 |
| Self-awareness | 0.8 | 0.2 | 0 | 1 |
| Empathy | 0.7 | 0.3 | 0 | 1 |
| Compassion | 0.6 | 0.4 | 0 | 1 |
| Kindness | 0.9 | 0.1 | 0 | 1 |
| Generosity | 0.8 | 0.2 | 0 | 1 |
| Humility | 0.7 | 0.3 | 0 | 1 |
| Patience | 0.9 | 0.1 | 0 | 1 |
| Forgiveness | 0.8 | 0.2 | 0 | 1 |
| Gratitude | 0.7 | 0.3 | 0 | 1 |
| Optimism | 0.9 | 0.1 | 0 | 1 |
| Positivity | 0.8 | 0.2 | 0 | 1 |
| Hope | 0.7 | 0.3 | 0 | 1 |
| Confidence | 0.9 | 0.1 | 0 | 1 |
| Self-esteem | 0.8 | 0.2 | 0 | 1 |
| Self-efficacy | 0.7 | 0.3 | 0 | 1 |
| Self-compassion | 0.9 | 0.1 | 0 | 1 |
| Self-acceptance | 0.8 | 0.2 | 0 | 1 |
| Self-actualization | 0.7 | 0.3 | 0 | 1 |
| Personal Growth | 0.9 | 0.1 | 0 | 1 |
| Life Satisfaction | 0.8 | 0.2 | 0 | 1 |
| Well-being | 0.7 | 0.3 | 0 | 1 |
| Quality of Life | 0.9 | 0.1 | 0 | 1 |
| Health-related Quality of Life | 0.8 | 0.2 | 0 | 1 |
| Psychological Well-being | 0.7 | 0.3 | 0 | 1 |
| Social Well-being | 0.9 | 0.1 | 0 | 1 |
| Environmental Well-being | 0.8 | 0.2 | 0 | 1 |
| Overall Well-being | 0.7 | 0.3 | 0 | 1 |